THE EMPLOYMENT OF URIC ACID SYNTHETIC MEDIUM FOR THE DIFFERENTIATION OF B. COLI AND B. AEROGENES

STEWART A. KOSER

From the Sheffield Laboratory of Bacteriology and Hygiene, Yale University

In the course of an investigation on the utilization of simple nitrogenous compounds of definite chemical composition, it was observed that B. aerogenes was able to multiply and grow luxuriantly in a medium which contained uric acid as the only source of nitrogen. B. coli, on the other hand, failed to develop. Because of the recent interest attached to these 2 types this point was investigated further, and a number of different strains of each organism was subjected to this test.

Several chemically definite mediums were employed. The most satisfactory results were obtained with the following:

Distilled ammonia-free water	1,000 c c.
NaCl	5.0 gm.
$MgSO_4$	0.2 gm.
$CaCl_2$	0.1 gm.
K_2HPO_4	1.0 gm.
Glycerol	30.1 gm.
Uric acid	0.5 gm.

This combination gives a colorless and clear medium. It was filled into ordinary test tubes and sterilized in the autoclave at 13-15 lbs. extra pressure for 15 minutes. A slight turbidity was apparent after autoclaving, due presumably to a finely divided precipitate of calcium sulphate. On cooling, the solution became clear.

In this medium B. aerogenes grew luxuriantly and soon produced a dense clouding. B. coli failed to develop and the tubes remained clear. In all, 124 different cultures of both B. coli and B. aerogenes were obtained and their ability to grow in the above medium tested. For these cultures the writer is indebted to Mr. C. C. Chen of this laboratory. The cultures of the colon bacillus were of fecal origin, while those of B. aerogenes had been isolated from various soils. Only those strains which were typical of either type were employed. At the same time these strains were also inoculated into the peptone-dextrose-

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dipotassium phosphate medium of Clark and Lubs.[1] The results of the methyl red and Voges-Proskauer tests in this medium were compared with the ability to develop in the uric acid solution. A striking correlation was found, which is summarized in the accompanying table.

TABLE 1

INCUBATED 4 DAYS AT 37 C.

Cultures Employed		Uric Acid Medium		Methyl Red Test		Voges-Proskauer Reaction	
		Growth	No Growth	Positive	Negative	Positive	Negative
B. coli	74	0	74	72	2	0	74
B. aerogenes	50	50	0	0	50	50	0

Within the first 24 hours the cultures of B. aerogenes attained a fair degree of turbidity and could easily be distinguished from the clear tubes which had been inoculated with the colon bacillus. As the cultures grew older the increased luxuriance of B. aerogenes accentuated this difference and the contrast was more striking.

The basic potassium phosphate is necessary to dissolve the uric acid, since this substance is insoluble in water. The glycerol also seems to aid in its solution. After the addition of the uric acid, the P_H value, as determined by the colorimetric method, was found to be 6.7-6.8. This slight acidity prevents the magnesium and calcium from precipitating as phosphates. In an effort to simplify the medium the magnesium sulphate and calcium chlorid were omitted. As the B. aerogenes cultures grew less luxuriantly in the absence of these salts this modification was discarded.

On the addition of 1.5% of washed shred agar to the solution mentioned in the foregoing an agar medium was obtained on which the same distinction between the 2 types may be brought out. Hypoxanthin hydrochlorid, when substituted for the uric acid, gave the same differentiation, although B. aerogenes appeared to grow less luxuriantly than in the uric acid medium. As the amount of available hypoxanthin was limited, a few tests only were performed.

Certain precautions were taken to exclude extraneous sources of nitrogen from the synthetic medium. All tubes were inoculated very lightly from 24-hour agar cultures. In addition, an effort was made to prevent, as far as possible, the absorption of gaseous ammonia by the medium, since B. coli, as well as B. aerogenes, is capable of grow-

[1] Jour. Inect. Dis., 1915, 17, p. 160.

ing in the presence of an ammonium salt, such as ammonium phosphate.

The distinction between these 2 types would seem to be due to the fact that B. aerogenes is capable of attacking the purin ring and utilizing the nitrogen thereof, while B. coli lacks this power and, since there is no source of available nitrogen, fails to develop. This differentiation is of interest in that it is one of fundamental nitrogen nutrition and not of carbohydrate metabolism, as are those distinctions based on the CO_2: H ratio, the attainment of a certain hydrogen-ion concentration, or the production of acetyl methyl carbinol.